REMARKS

The above amendments to the above-captioned application along with the following remarks are being submitted as a full and complete response to the Official Action dated September 13, 2004.

Applicants respectfully request the Examiner to acknowledge the receipt of the IDS filed on August 26, 2004 in the next office action.

In view of the above amendments and the following remarks, the Examiner is respectfully requested to give due reconsideration to this application, to indicate the allowability of the claims, and to pass this case to issue.

Status of the Claims

Claims 1-4 and 6-14 are under consideration in this application. Claims 1, 3-4, 11-12 and 14 are being amended, as set forth above, in order to more particularly define and distinctly claim Applicants' invention. Applicants hereby submit that no new matter is being introduced into the application through the submission of this response.

Formality Rejection

Claims 1 - 4 and 6 - 14 were rejected under 35 U.S.C. § 112, second paragraph, as being vague and indefinite. As indicated, the claims have been amended as suggested or required by the Examiner. Accordingly, the withdrawal of the outstanding informality rejections is in order, and is therefore respectfully solicited.

Prior Art Rejections

Under 35 U.S.C. § 102(b), claims 1 - 4, 6 - 9, 11, 12 and 14 were rejected as being anticipated by WO 98/28440 to Nyren (hereinafter "Nyren"), and claims 1, 3, 4, 6, 7 and 9 were rejected as being anticipated by the article of Nordstrom et al (Analytical Biochemistry (2000) 282:186-193). (hereinafter "Nordstrom"). Under 35 U.S.C. § 103(a), claims 10 and 13 were rejected as being unpatentable over Nyren in view of the article of Ishikawa et al (Human Immunology (1995) 42:315-318, hereinafter "Ishikawa"). These rejections have been carefully considered, but are most respectfully traversed.

The present invention as now recited in claim 1 is directed to a method of analysis of DNA sequence, comprising the steps of: pretreating a solution containing a nucleic acid substrate for a complementary strand extension reaction by degrading, using pyrophosphatase, pyrophosphoric acid contained in the solution, and/or degrading, [[by]] using apyrase, adenosine 5'–triphosphate contained in the solution; removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the pretreating step; mixing the solution with a DNA primer, a target nucleic acid and a reagent for the extension reaction on the DNA primer after the step of removing or inactivating; conducting the extension reaction on the DNA primer hybridized to the target nucleic acid; and detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step.

The present invention as recited in claim 3 is now directed to a method of analysis of DNA sequence, comprising steps of: adding pyrophosphatase and/or apyrase to one or more solutions each containing a different deoxynucleotide, or an analogue of the deoxynucleotide and then thereby degrading pyrophosphoric acid or adenosine 5'–triphosphate, respectively, contained in the solutions; removing or inactivating the pyrophosphates and/or the apyrase in the solution after the step of degrading after the adding step; mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating; and extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions; and detecting pyrophosphoric acid generated during an extension reaction by chemiluminescence-reaction after the removing or inactivating step.

In addition, according to claim 4, the present invention is directed to a method of analysis of DNA sequence comprising steps of: adding pyrophosphatase to one or more solutions each containing a different deoxynucleotide, or an analogue of the deoxynucleotide and then thereby degrading pyrophosphoric acid contained in the solutions; removing or inactivating the pyrophosphates in the solutions after the step of degrading after the adding step; mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for an extension reaction of the DNA primer after the step of removing or inactivating the pyrophosphatase; extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions and

converting pyrophosphoric acid, generated during the extension reaction, into adenosine 5'-triphosphate in presence of adenosine 5'-phosphosulfate and ATP sulfurylase; and detecting luminescence caused by chemiluminescence-reaction using the adenosine 5'-triphosphate, a luminescence-enzyme and a luminescence substrate after the removing or inactivating step.

Further, as recited in claim 11, the present invention is directed to a method of analysis of DNA sequence, comprising steps of: a first step of adding pyrophosphatase to each of a solution containing deoxyadenosine 5'-α-thiotriphosphate, a solution containing deoxythymidine 5'-triphosphate, a solution containing deoxyguanosine 5'-triphosphate and a solution containing deoxycytidine 5'-triphosphate, and then thereby degrading pyrophosphoric acid contained in each of the solutions; a second step of removing or inactivating the pyrophosphatase in each of the solutions after the first step; a third step of mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the second step; a fourth step of extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid generated during the extension reaction into adenosine 5'-triphosphate in presence of adenosine 5' phosphosulfate and ATP sulfurylase; and a fifth step of detecting luminescence caused by chemiluminescence-reaction using the adenosine 5' triphosphate, lusiferase and luciferin after the second step.

Even more, the present invention as recited in claim 12 is directed to a method of analysis of DNA sequence, comprising steps of: a first step of adding pyrophosphatase to a solution containing deoxyadenosine 5'-α-thiotriphosphate, deoxythymidine 5'-triphosphate, deoxyguanosine 5'-triphosphate and deoxycytidine 5'-triphosphate, thereby degrading the pyrophosphoric acid contained in the solution; a second step of removing or inactivating the pyrophosphatase in each of the solutions after the first step; a third step of mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for an extension reaction of the DNA primer after the second step, and a fourth step of extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid, generated during the extension reaction, into adenosine 5'-triphosphate in presence of adenosine 5' phosphosulfate and ATP sulfurylase; and a

fifth step of detecting luminescence caused by chemiluminescence-reaction using the adenosine 5' triphosphate, lusiferase and luciferin after the second step.

Among the main features of the present invention as recited in at least the above-noted claims, the method of the invention incorporates pretreating a solution containing a nucleic acid substrate for complementary strand extension reaction by degrading, using pyrophosphatase and/or apyrase, pyrophosphoric acid or adenosine 5'– triphosphate, respectively, contained in the solution"; "removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of pretreating"; "mixing the solution, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating"; and detecting pyrophosphoric acid or adenosine 5'– triphosphate generated as a result of the extension reaction. Pretreatment with pyrophosphatase enables most of pyrophosphoric acid to be degraded and thus the luminescence signal intensity of background noise to be sufficiently negligible, as shown in Fig. 4 (p. 28, lines 3-6).

Applicants will point out that that dNTPs generate PPi (pyrophosphoric acid) by thermal degradation or the like and thus generate the largest noise signal (p. 27, line 16; p. 19, lines 3-7; "*The solution containing the single stranded DNA 104 is then pretreated by adding thereto apyrase 105 and PPase 106, whereby dNTPs and PPi remaining in the solution are degraded. These enzymes such as Apyrase 105 and PPase 106 will disturb the subsequent measurement so that they are removed from the solution (107)*" p. 20, lines 9-15). Depending on the reagent supplied by different companies with different manufacturing methods, different lots and different storage conditions, the amounts of PPi/impurities in dNTPs or analogies differ (p. 27, lines 15-18) thus creating different amount of background noise. By pretreating the solution and then removing or inactivating the pyrophosphatase or apyrase therefrom, the invention significantly reduces the noise caused by PPi and/or ATP generated by thermal degradation of the nucleic acid substrate dNTPs ("*In high sensitivity measurement, these impurities must be degraded and removed from the reagent.*" p. 9, lines 7-9; p. 21, lines 5-18), such that PPi generated by the later extension reaction can be detected with a high degree of sensitivity, as illustrated by Figs. 4-5 ("*FIG. 4 illustrates comparison of noise signals between presence and absence of pretreatment with PPase in Embodiment 1 of the present*

invention; FIG. 5 illustrates dependence of noise signals on the concentration of dCTP after pretreatment with PPase in Embodiment 1 of the present invention", p. 16, 27-28).

Applicants respectfully contend that none of the cited prior art references teaches or suggests the combination of pretreating a solution containing a nucleic acid substrate for complementary strand extension reaction by degrading, using pyrophosphatase and/or apyrase, pyrophosphoric acid or adenosine 5'– triphosphate, respectively, contained in the solution"; "removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of pretreating"; "mixing the solution, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating"; and detecting pyrophosphoric acid or adenosine 5'– triphosphate generated as a result of the extension reaction, as recited for present the invention.

In contrast, Nyren merely discloses a method of identifying a base at a target position in a sample DNA sequence by detecting pyroposphate (PPi) enzymically (See Abstract). The reference discloses using a "nucleotide-degrading enzyme" including all enzymes capable of non-specifically degrading nucleotides (page 3, last line – page 4, line 6). This nucleotide-degrading enzyme immobilized on solid may be added after nucleotide incorporation has taken place and may be removed from the reaction mixture before the next nucleotide is added (page 6, lines 14-24).

On the other hand, the present invention uses pyrophosphatase for degrading PPi before "mixing the solution, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating" (See p. 7, lines 10-15; claim 1). As PPi is different from the nucleotide, pyrophosphatase is different from "nucleotide-degrading enzyme". In other words, among other distinctions, the present invention differs from Nyren in (1) the kinds of the enzyme, and (2) the timing of adding or removing associated with the intended reactions or methods. Moreover, though Nyren describes the use of pyrophosphatase (p. 19, lines 2-9), the reference does not disclose, teach or suggest how and/or when the pyrophatase is used in a manner similar to that of the pressent invention. Thus, Nyren fails to disclose or suggest each and every element claimed for the present invention, and thus cannot anticipate the present invention.

The reference to Nordstrom merely describes pyrosequencing with enzymatic strategies for template preparation (See Abstract). This reference shows incubating the PCR-ampified product with different combinations of enzymes, such as pyrophosphatase ("Enzymatic Preparation of dsDNA Templates", page 187). However, Nordstrom does not show or suggest of the use of pyrophosphatase for the deoxynucleotide triphosphate which is used for pyrosequencing ("Pyrosequencing" in page 187). Thus, Nordstrom also fails to show or suggest each and every feature of the claimed invention, and thus cannot anticipate the present invention as claimed.

With respect to the rejection under 35 USC §103(a), as explained above, Nyren by itself fails to anticipate each and every feature of the claimed invention. Further, Applicants will contend that this reference by itself also fails to provide any disclosure or suggestion that would render each and every feature of the claimed invention obvious to one of skill in the art. The secondary reference to Ishikawa merely describes the use of primers for detecting a single base difference between A2 alleles and other HLA-A alleles, having one extra mismatch at the second position from its 3'-end (See Abstract). This reference fails to provide any teaching or suggestion that would make up for the deficiencies in Nyren such that their combination could render the features of the present invention obvious. In other words, even if these two references were combined, that combination would still fall short of embodying the combination of pretreating a solution containing a nucleic acid substrate for complementary strand extension reaction by degrading, using pyrophosphatase and/or apyrase, pyrophosphoric acid or adenosine 5'– triphosphate, respectively, contained in the solution"; "removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of pretreating"; "mixing the solution, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating"; and detecting pyrophosphoric acid or adenosine 5'– triphosphate generated as a result of the extension reaction. Thus, the present invention as claimed cannot be rendered obvious in view of Nyren and Ishikawa.

Conclusion

In view of all the above, clear and distinct differences as discussed exist between the present invention as now claimed and the prior art references upon which the rejections in the

Office Action rely, Applicants respectfully contend that the prior art references cannot anticipate the present invention or render the present invention obvious. Rather, the present invention as a whole is distinguishable, and thereby allowable over the prior art.

Favorable reconsideration of this application is respectfully solicited. Should there be any outstanding issues requiring discussion that would further the prosecution and allowance of the above-captioned application, the Examiner is invited to contact the Applicants' undersigned representative at the address and phone number indicated below.

Respectfully submitted,

Stanley P. Fisher
Registration Number 24,344

Juan Carlos A. Marquez
Registration Number 34,072

REED SMITH LLP
3110 Fairview Park Drive
Suite 1400
Falls Church, Virginia 22042
(703) 641-4200

December 13, 2004